Clinical Effects of a Proprietary Combination Isoflavone Nutritional Supplement in Menopausal Women: A Pilot Trial

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Background • As they reach menopause, a majority of women living in Westernized countries experience climacteric symptoms. Hormone replacement therapy (HRT) has been used to remediate these symptoms. Recent studies, however, have suggested that HRT may increase the risk of developing breast cancer and cardiovascular disease (CVD). Therefore, many women are looking for alternative treatment options.

Purpose • This trial was a pilot study to assess the effect of a nutritional supplement containing isoflavones from kudzu and red clover, along with other targeted nutrients on menopausal symptoms and markers of breast cancer and CVD risk. Twenty-five menopausal women suffering from severe hot flushes and night sweats completed a 12-week intervention using this combination isoflavone nutritional supplement.

Results • We observed a 46% decrease in reported hot flushes, from an average of 9.7 to 5.2 per day. Quality of life, as assessed by the standardized Greene Questionnaire, showed similar improvement. Two markers of CVD risk, the ratio of total cholesterol to high-density lipoprotein (HDL) cholesterol and homocysteine, showed modest improvement. A proposed marker of breast cancer risk, the ratio of 2-hydroxyestrone to 16 alpha-hydroxyestrone, also showed a statistically significant improvement.

Conclusions • The results of this pilot trial suggests that this combination isoflavone nutritional supplement may significantly relieve the most troubling symptoms of menopause, as well as confer some chemopreventive and cardioprotective benefits.

Institution in which the work was performed • Functional Medicine Research Center, Gig Harbor, Wash, a division of Metagenics, Inc.

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Women often experience climacteric symptoms as they reach menopause. These symptoms can vary in intensity and frequency, but most often include hot flushes, night sweats, and vaginal dryness.

Estimates of the number of menopausal women who experience these symptoms vary widely, with some studies suggesting that 75% to 85% of women in Western countries report menopausal complaints, while rates in Asian countries are as low as 15%.1,2

Hormone replacement therapy (HRT) is recognized as the most effective treatment for the relief of short-term symptoms of menopause. Recent findings, however, suggest that HRT may increase the long-term risks for breast cancer, thromboembolism, and cardiovascular disease (CVD).3 Additionally, many women taking HRT experience unpleasant side effects, such as vaginal bleeding, breast tenderness, and bloating. It is therefore not surprising that the overall compliance using this therapy is <20%.4,5 Clearly, the early promise of HRT as a panacea for menopausal women has not been realized.

Plants containing isoflavones have traditionally been used in Asian countries for the relief of climacteric symptoms. Isoflavones are found in soy, kudzu, and red clover, and have been shown to bind to estrogen receptors and exert weak estrogenic as well as antiestrogenic effects. Because of their ability to both positively and negatively influence estrogen activities, isoflavones have been called nature’s selective estrogen receptor modulators (SERMs).6
A number of trials assessing menopausal symptoms have used either foods high in isoflavones or the isolated extracts themselves. Data are equivocal, with some positive and some negative results. These inconsistent findings may be due to a variety of variables: variation in the populations studied, type and amount of isoflavones used, length and design of the trial, and apparent individual variability to the physiologic effects of isoflavones. Although definitive conclusions on the efficacy of isoflavones in climacteric complaints are not available, women use these foods and extracts in increasing numbers. With the controversy surrounding HRT, many women appear willing to compromise for more modest symptom reduction using natural compounds. Clearly, while menopausal symptoms can significantly affect quality of life, women of this age group are equally concerned about the long-term risks of CVD and breast cancer.

In this pilot trial, we assessed the effect of a combination isoflavone nutritional supplement with targeted nutrients on both climacteric symptoms in menopausal women and markers of CVD and breast cancer risk.

METHODS

Subject Criteria

The study was performed at the Functional Medicine Research Center (FMRC), Gig Harbor, Wash, from January to May 2002. Potential subjects were recruited through newspaper and radio advertisements. Women aged 40 to 65 years with either 6 months of amenorrhea and a biochemical criterion for menopause (ie, follicle-stimulating hormone [FSH] >50 mIU/mL, estradiol [E2] <20 pg/mL) or 12 months of amenorrhea (with or without biochemical criterion) were accepted for the trial. Subjects younger than 40 were eligible to participate only if they had had a complete bilateral ovariectomy more than 6 weeks before the beginning of the trial. Inclusion criteria included experiencing a combination of more than 40 hot flushes per day (an average of 6 hot flushes per day). Individuals were excluded from participating in the trial if they had evidence of an untreated endocrine, neurological, or infectious disorder; pregnancy or lactation; history of diabetes; liver, kidney, or heart disease; history of mental illness or attempted suicide; use of oral corticosteroids within 4 weeks of screening; use of oral contraceptives, oral estrogens, or estrogen-, progestin-, or progesterone-containing creams or patches; active cancer or a personal history of cancer (excluding skin cancer); use of a supplement containing isoflavones in the preceding 4 weeks; or evidence of human immunodeficiency virus or acquired immunodeficiency syndrome. The initial screening visit included a laboratory assessment to exclude women with abnormal complete blood count (CBC), glucose, kidney, and/or liver function.

STUDY DESIGN

The Washington Institutional Review Board (Olympia, Wash) approved the study protocol. Candidates who agreed to participate signed informed consent forms. The clinical trial was a single-arm, open-label, observational study. All subjects completed a 2-week run-in (control) period, during which they kept daily records of the number and intensity of hot flushes. At 2 weeks, they returned to start the active phase of the trial. Subjects who showed an average of fewer than 40 hot flushes per week during the run-in period were disqualified from participation.

Each participant was dispensed a 90-count bottle of the nutritional supplement (Estrofactors, Metagenics Inc, San Clemente, Calif) and was instructed to take 3 tablets once a day with food. The supplement’s ingredients are listed in Table 1. Subjects were also instructed to maintain their customary dietary and lifestyle patterns. Diet and lifestyle habits were monitored using questionnaires at the beginning of the study, and at 6, 10, and 14 weeks to identify any changes. Blood pressure, pulse, and weight were collected at screening, 2, 6, 10, and 14 weeks. Compliance was calculated by tablet count of the returned containers and by before-and-after serum isoflavone measurements.

A change in the frequency and intensity of hot flushes by self-report on daily symptom records was the primary endpoint assessed. The average frequency and intensity of hot flushes per day during the 2-week control period was taken as baseline and compared to the average of the last 2 weeks of the treatment period.

Secondary clinical endpoints included assessment of subjective improvement of menopausal symptoms, measured using the Greene Climacteric Questionnaire. The questionnaire is a standardized menopause-specific instrument, which measures symptoms of the climacteric including hot flushes and night sweats. Data were collected at screening, 2, 6, 10, and 14 weeks. Fasting blood samples were taken at the beginning and end of the study to assess for changes in liver and kidney function, glucose, and CBC. Additional laboratory tests included FSH (taken at the beginning of the trial only); estrogens (estrone [E1], [E2], estriol [E3]; 2-hydroxyestrone [2-OHE]; 16 alpha-hydroxysterone [16alpha-OHE]); progesterone; testosterone; sex hormone binding globulin (SHBG); dehydroepiandrosterone-sulfate (DHEA-S); homocysteine; blood lipids; and isoflavones.

Analytical Methods

Aspartate aminotransferase, alanine aminotransferase, bilirubin, urea nitrogen, creatinine, albumin, and glucose were assessed by standard photometric methods; CBC was assessed by Coulter GEN-S (Beckman Coulter, Inc, Fullerton, Calif); FSH and E2 were assessed by chemiluminescence; triglycerides, total cholesterol high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol (cholesterol, HDL-C, and LDL-C, respectively) were determined by photometric analysis; and plasma homocysteine was assessed by high-performance liquid chromatography at Laboratories Northwest (Tacoma, Wash). Radioimmunoassay measurements of SHBG, progesterone, testosterone, DHEA-S, E1, E2, and E3, and enzyme-linked immuno- sorbent assay (ELISA)-colorimetric analysis of 2-OHE and 16-
alpha-OHE were performed by Great Smokies Diagnostic Laboratories (Asheville, NC). Gas chromatography (GC)/mass spectrometry measurements of the isoflavones (daidzein, genistein, equol, glycitein, O-MDA, formononetin, and biochanin A) were performed in the laboratory of Kenneth D. R. Setchell, PhD, Children’s Hospital Medical Center (Cincinnati, Ohio).

Statistical Analysis

Data were analyzed by a one-way analysis of variance (ANOVA) using the JMP Statistical Package (SAS Institute, Cary, NC). Variances in laboratory analyses were determined using multiple split samples. Average values are presented as mean ± standard error of the mean (SEM).

RESULTS

One hundred and eighty women were initially screened; of these, 31 women were accepted for the study. Twenty-five of the 31 subjects (average age 53 years) completed the trial. Six participants dropped out before completion, 5 because of an inability to comply with the study protocol and 1 because the subject did not fit the flush criteria after the initial 2-week run-in period. All subjects on screening had normal CBC, serum glucose, and liver and kidney function. No statistically significant changes in the screening laboratory tests were noted at the conclusion of the trial (data not shown). No significant changes in weight and blood pressure were observed throughout the trial (data not shown). Tablet count and serum isoflavones measured before and after supplementation suggested good compliance with the protocol (data not shown).

Both the frequency and intensity of hot flushes decreased significantly when the initial run-in values were compared to occurrence during the last 2 weeks of the 12-week intervention. Frequency decreased from an average of 69 ± 5 hot flushes per week to 37 ± 6 hot flushes per week, for an average decrease of 46% (P<.001; Figure 1). The data obtained from the Greene Climacteric Questionnaire supported this observation. The category of vasomotor symptoms on the Greene Climacteric Questionnaire significantly decreased from a score of 4.8 ± 0.2 to 3.1 ± 0.3 (P<.001). As shown in Figure 2, all categories of the Greene Climacteric Questionnaire, including psychological, somatic, anxiety, and depression, significantly decreased, and the overall score was significantly reduced from 20 ± 1.4 to 14 ± 1.4 (P<.001).

Homocysteine and the chol:HDL-C ratio also showed a significant decrease. While total cholesterol did not significantly decrease over the course of the intervention, the chol:HDL-C ratio decreased from 4.71 ± 0.35 to 4.32 ± 0.29 (P<.05), for an overall decrease of 8% among all participants. The decrease in the ratio was even greater when the participants were stratified by presentation below or above 4.0 chol:HDL-C. Homocysteine decreased from an initial average of 8.29 ± 0.32 pg/mL to 7.51 ± 0.25 pg/mL (P<.005). However, when the homocysteine data are analyzed only for those subjects who initially presented with elevated homocysteine (>8.0 pg/mL), the resulting data are more dramatic. Initially, 14 of the subjects had homocysteine values greater than 8.0 pg/mL, and 8 of these subjects had greater than 9.0 pg/mL. After the intervention, homocysteine values were reduced to less than 9.0 pg/mL in all but 1 subject, and 7 of these had reductions to 8.0 pg/mL or lower (Table 2). Figure 3 shows the relationship between the stratified chol:HDL-C ratio (above or below 4.0) and the stratified homocysteine data (above or below 8 pg/mL) at the start and finish of the trial.

Recent research has suggested the ratio of 2-OHE:16 alpha-OHE may be valuable as a risk factor for breast cancer.18-20 We analyzed serum for 2-OHE and 16 alpha-OHE. The initial 2-OHE and 16 alpha-OHE values were 140 ± 6.20 pg/mL and 315 ± 11.0 pg/mL, respectively. After the intervention with the combination isoflavone nutritional supplement, 2-OHE was significantly increased to 209 ± 13.7 pg/mL (P<.01), whereas 16 alpha-OHE was significantly decreased to 296 ± 13.7 pg/mL (P<.05). The change in these values resulted in a significant increase in the ratio of 2-OHE:16 alpha-OHE from 0.46 ± 0.024 initially to 0.71 ± 0.063 (P<.001), a 35% increase (Figure 4).

DISCUSSION

Women who reach menopause face a number of issues. Although many go to their doctors seeking treatment for climacteric symptoms, longer-term questions of heart disease and breast cancer are often underlying concerns. The negative results from the recent Women’s Health Initiative trial have put many menopausal women in a quandary.3 It appears that HRT has failed to achieve the early promise of a safe therapy, and women are searching for alternative approaches.
We have developed a combination isoflavone nutritional supplement with natural ingredients aimed at providing relief for women's menopausal symptoms as well as promoting healthy cardiovascular function. During the 12-week intervention with this nutritional supplement, we observed a significant decrease in reported hot flushes from an average of 9.7 per day to 5.2 per day. Quality of life, as assessed by the Greene Questionnaire, also improved. All categories on the questionnaire's individual subscales (psychological, somatic, vasomotor, anxiety, and depression) showed a statistically significant improvement (Figure 2). Data have been reported on the effect of isoflavones from soy and red clover alone on attenuating hot flushes in menopausal women; however, this is the first trial to look at the effects of a combination isoflavone product made with kudzu and red clover for the remediation of hot flushes.

During the past decade, many investigators have reported a beneficial effect of soy isoflavones on menopausal symptoms, with decreases averaging around 40% to 50%, although some studies have found no response. For example, Albertazzi et al reported that 60 g/d isolated soy protein reduced hot flushes by 45% versus a reduction of 30% with placebo (60 g/d casein) over 12 weeks in 104 postmenopausal women. Red clover isoflavones also have been reported to remediate hot flushes, with positive studies showing reductions of 40% to 54%. The purified isoflavone genistein also has been reported to significantly reduce hot flush symptoms as compared to placebo in postmenopausal women at a dosage of 54 mg/d over 1 year. The approximately 50% improvement in symptoms in our study agrees with the published studies using soy or red clover isoflavones. Although our study did not have a control group, the placebo controls in published studies have averaged from 20% to 30%, which suggests the magnitude of effect in this trial is unlikely to be entirely due to placebo. In fact, the North Central Cancer Treatment Group, which has studied more than 1,000 patients with hot flush symptoms over 10 years, has suggested that a reduction of hot flush activity of at least 45% in a pilot trial indicates that it is reasonable to pursue further testing of an agent in a larger-phase, randomized trial.

Several markers of CVD risk also showed improvement over the 12-week intervention. The chol:HDL-C ratio significantly improved. This observation may be attributed to the isoflavones in the product, as some studies have suggested isoflavones may exert a modest, positive effect on the chol:HDL-C ratio. Serum levels of homocysteine also improved. Homocysteine decreased an average of 9% in the whole group. In women who started with elevated levels (>8 pg/mL), a more significant 13% decrease was observed. Epidemiological studies have shown that higher blood homocysteine levels appear to be associated with higher risks of coronary, cerebral, and peripheral vascular disease and are inversely related to blood levels of folate and vitamins B₁₂ and B₆.

Research suggests that these vitamins may offer value in breast cancer risk by influencing the catechol-O-methyltransferase (COMT) enzyme as well. COMT catalyzes the O-methylation of the catechol estrogens, and several studies have

### TABLE 2 Changes in Blood Homocysteine Levels for Subjects With Initial Homocysteine Levels Greater Than 8 pg/mL (n=14).

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### FIGURE 2 The mean (± SEM) standardized Greene Questionnaire scores for the subjects (N=25) before (start, shaded bars) and after (finish, clear bars) the 12-week intervention with the combination isoflavone nutritional supplement. Significant decreases were observed in all categories: psychological (psych; P < .001), somatic (P < .05), vasomotor (vaso; P < .001), anxiety (P < .005), and depression (P < .005).
suggested a relationship between COMT polymorphisms that can cause a 3- to 4-fold decrease in this methylation with an increased risk to breast cancer. These findings indicate a role for certain folate pathway micronutrients in mediating the association between COMT genotype and breast cancer risk.

We observed a significant increase in the 2-OHE:16 alpha-OHE ratio. Research suggests that women who metabolize a larger proportion of their estrogens through the C-16 pathway, as opposed to the C-2 pathway, have an elevated breast cancer risk. For example, in a trial of 10,786 premenopausal women followed for 5.5 years, it was found that participants with increased levels of 2-OHE had a 40% decrease in the occurrence of breast cancer. A longer-term study on postmenopausal women showed those with the highest 2-OHE:16 alpha-OHE ratio had a 30% lower risk of developing breast cancer than women with a lower ratio. Although not all studies have been positive, the data overwhelmingly indicate that a higher 2-OHE level is beneficial, especially in postmenopausal women at risk for developing hormone-dependent cancer.

While much of the research on the 2-OHE:16 alpha-OHE ratio has focused on the phytonutrient indole-3-carbinol, which is found in cruciferous vegetables, the increase in our trial may be due to the isoflavones. In studies on both pre- and postmenopausal women, it has been shown that isoflavones increase the beneficial 2-OHE at the expense of the 16 alpha-OHE, resulting in an increase in the 2-OHE:16 alpha-OHE ratio. Moreover, it may be that the specific isoflavones found in kudzu have the most pronounced effect. One of kudzu’s isoflavones, puerarin, induces the cytochrome P450 enzymes 1A1 and 1A2; these enzymes are instrumental in increasing 2-hydroxylation of estrogens. Additionally, preliminary research suggests that the herb rosemary (*Rosmarinus officinalis*), also an ingredient in the supplement, may promote 2-hydroxylation of estrogen and support an increased 2-OHE:16 alpha-OHE ratio as well.

CONCLUSION

This observational study suggests that a combination isoflavone nutritional supplement may help to reduce hot flushes. In addition, a modest but statistically significant improvement in the 2-OHE:16 alpha-OHE ratio, chol:HDL-C ratio, and homocysteine levels suggests that this combination nutritional formula may potentially confer not only symptomatic but some chemopreventive and cardioprotective effects for women in menopause. A placebo-controlled trial to follow up on these initial observations is warranted.

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References